



## Aroma characterization and *in vitro* antihypertensive activity of Amazonian Camu-camu (*Myrciaria dubia*) fruit

Juliana María García-Chacón<sup>a\*</sup>, Diana Paola Forero<sup>b</sup>, Devin G. Peterson<sup>b</sup>  
and Coralia Osorio<sup>a\*</sup>

<sup>a</sup>Departamento de Química, Universidad Nacional de Colombia, AA 14490, Bogotá, Colombia

<sup>b</sup>Department of Food Science and Technology, Parker Food Science & Technology Building, The Ohio State University, 2015 Fyffe Rd., The Ohio State University, Columbus, Ohio 43210, United States

\*Corresponding author: Coralia Osorio and Juliana García-Chacón, Universidad Nacional de Colombia, AA 14490 Bogotá, Colombia. Tel: +57-6013165000, ext. 14472; E-mail: cosorior@unal.edu.co and jumgarcia@unal.edu.co

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### Abstract

The Amazonian fruit known as camu-camu (*Myrciaria dubia*) has recently attracted attention due to its sensory and biofunctional properties. A sensomics methodology was used to identify the odour-active volatiles of the whole fruit (pulp and peel) by using solvent-assisted flavour evaporation (SAFE), analysis by gas chromatography-mass spectrometry (GC-MS), and gas chromatography-olfactometry (GC-O), and sensory evaluation (aroma extract dilution analysis (AEDA)) techniques. Four odour-active volatile compounds were reported at concentrations above the odour-activity values: isoamyl acetate,  $\alpha$ -pinene, limonene, and  $\beta$ -caryophyllene. Recombination aroma testing further verified that the compounds contributed to the fruity, herbal, citrus, and woody notes of the *M. dubia* fruit. Furthermore, the fruit *in vitro* ACE-I inhibition activity was  $39.48 \pm 12.09\%$  (at 50  $\mu\text{g}/\text{mL}$ , using lisinopril as positive control), showing a potential use as a functional food.

**Keywords:** Odour-active volatiles; GC-O; SAFE; Antihypertensive activity; Terpenes.

### 1. Introduction

Camu-camu (*Myrciaria dubia*) is a Myrtaceae berry from the Amazon Rainforest. Consumption of the fruit has been related to health-promoting benefits due to the presence of bioactive compounds such as carotenoids, polyphenols (anthocyanins, flavones, and flavonols), and malic acid, as well as its high vitamin C content (Neves et al., 2015; Cunha-Santos et al., 2019). Specific biological activities of the fruit associated with the prevention of metabolic syndrome diseases include antihypertensive and antihyperglycemic activities by the inhibition of angiotensin-converting enzyme-I (ACE-I) and  $\alpha$ -amylase and  $\alpha$ -glucosidase enzymes (responsible for the hydrolysis of polysaccharides in the human intestine) have been respectively reported (Fidelis et al., 2018; García-Chacón et al., 2022). In fact, as part of traditional medicine, Amazonian indigenous communities consumed camu-camu fruit for the treatment of some diseases such as diabetes,

influenza, hypercholesterolemia, inflammation, migraine, and cancer, in addition to using the fruit as a phytomedicine to counter secondary effects from cardiovascular diseases (CVDs) (Castro et al., 2020).

Hypertension is a medical condition suffered by 1.28 billion adults worldwide and is the primary cause of premature death from CVDs (WHO, 2023). In most cases, high blood pressure is treated with calcium channel blockers or by inhibiting the ACE-I enzyme (Angiotensin-converting-I enzyme), which prevents the conversion of angiotensin I to angiotensin II, reducing the high blood pressure and preventing renal insufficiency, along with cardiovascular and brain risks. Some common antihypertensive medications employed to reduce high blood pressure have caused side effects in patients, including effects on the immune system functions, dry cough, and angioedema (Felkle et al., 2022). Recent *in vitro* studies reported that camu-camu seed coat extracts, obtained with water, ethyl alcohol, and propanone, inhibited the ACE-I enzyme by 28–40% (Fi-

delis et al., 2018); however, neither the pulp nor the peel of the fruit of *M. dubia* has been investigated for antihypertensive activity.

Camu-camu has also been used in food formulation and processing, increasing its commercialization and sensory acceptance through food products such as cookies, yogurt, infusions, and candies (Santos et al., 2022). The first attempt to study the volatile composition of camu-camu fruit was made by Franco and Shibamoto (2000) on the headspace using a Porapak Q trap and further elution with hexane. The major constituents of this extract were terpenes (98%):  $\alpha$ -pinene, *d*-limonene, and  $\beta$ -caryophyllene. Later, Campelo et al. (2020) studied the changes induced by dielectric barrier discharge plasma processing on the volatile composition of camu-camu fruit. After the process, they obtained the volatile extract of camu-camu fruit by HS-SPME (Headspace-Solid Phase Microextraction) using a DVB/CAR/PDMS fiber. In addition, the most abundant constituents were terpenes (mono- and sesquiterpenes), followed by some monoterpenols. The most abundant volatile compounds extracted by HS-SPME were  $\beta$ -caryophyllene,  $\alpha$ -pinene, *d*-limonene,  $\alpha$ -terpineol,  $\alpha$ -humulene,  $\alpha$ -calacorene, and  $\gamma$ -cadinene. However, the most relevant volatile compounds in the aroma of this fruit have not been characterized. This can be done using the sensomics approach that combines the instrumental and sensorial (GC-O) analyses of a volatile-rich extract to locate the compounds that can interact with the odorant receptors using a bio-response (Granvogl & Schieberle, 2022).

The aim of this research was to determine the odour-active volatile compounds responsible for the distinctive aroma of camu-camu fruit, together with assessing the *in vitro* antihypertensive activity of the fruit.

## 2. Materials and methods

### 2.1. Chemicals

Dichloromethane (DCM) and anhydrous sodium sulfate ( $\text{Na}_2\text{SO}_4$ ) were purchased from Merck (Darmstadt, Germany). The following compounds were commercially obtained and used as reference odorants: *n*-alkane mix ( $\text{C}_7$ - $\text{C}_{30}$ ), linalool,  $\alpha$ -pinene, limonene,  $\beta$ -caryophyllene, and isoamyl acetate standards were acquired from Sigma-Aldrich (St. Louis, MO, USA); and *cis*-3-hexenol from TCI (Tokyo, Japan). Likewise, food-grade propylene glycol (Interpharma de Colombia S.A.S, Bogotá, Colombia) was employed for preparing the reference solutions for the sensory analysis. For *in vitro* ACE-I analyses, deionized water was obtained at a resistivity = 25  $\text{M}\Omega \cdot \text{cm}$  at 25 °C.

### 2.2. Fruit material

The fruits of *M. dubia* were purchased at the local markets of Florencia (Caquetá, Colombia) and carefully transported in freezing conditions the same day to Bogotá for their analyses. Fully ripe fruits (pH  $2.43 \pm 0.03$ , soluble solid content of  $7.38 \pm 0.18^\circ$  Brix, and acidity content of  $2.20 \pm 0.85\%$  citric acid) were selected, and the seeds were manually removed. All the extracts were obtained with the whole fruit (pulp and peel).

### 2.3. Determination of odor-active volatile compounds

#### 2.3.1. Isolation of volatile extract

A puree from whole camu-camu (pulp and peel, 180 g) was ob-

tained using a commercial stainless-steel blender. After adding 100 mL of DCM to the puree, anhydrous sodium sulfate (210 g) was gradually added with continuous stirring and cooling. Subsequently, the mixture was filtered through defatted cotton wool, and the combined organic phases were extracted by the Solvent Assisted Flavour Evaporation (SAFE) technique, following the methodology published by Engel et al. (1999). The organic phase was concentrated to 1 mL using a Vigreux column ( $50 \times 1$  cm). Finally, a concentrated and colorless extract exhibiting the characteristic *M. dubia* aroma was obtained.

#### 2.3.2. Chromatographic analyses

The SAFE extract was submitted to GC-MS and GC-O analyses using a 7890B gas chromatograph (Agilent Technologies Inc. Wilmington, DE, USA) coupled to an Agilent 5977A mass spectrometer, operated in electron impact mode (70 eV) with automatic injection and source temperatures of 250 °C, running in 2 GHz enhanced dynamic range mode. The spectral data were collected in profile at 10 Hz scan speed, and mass spectra were acquired in a mass range between 40 and 300 u and processed in ChemStation software (Rev. E01) with NIST library (version 2.2, NIST/EPA/NIH, 2014). Two capillary columns were used, a DB-WAX polar column ( $60 \text{ m} \times 0.25$  mm id, 0.25  $\mu\text{m}$ ) and a DB-5 semipolar column ( $60 \text{ m} \times 0.25$  mm id, 0.32  $\mu\text{m}$ ). The samples were injected in split mode (1:10), using Helium (2.0 mL/min) as the carrier. The DB-WAX column oven was programmed from 40 °C for 2 min to 180 °C, at 6 °C/min, then at 12 °C/min to 230 °C, and finally held at 230 °C for 7 minutes. Likewise, the DB-5 column oven was programmed from 60 °C for 6 min followed by a heating rate of 6 °C/min up to 180 °C for 15 min, and finally, the column was kept at 300 °C for 1 min. For GC-O analyses, the end of the capillary was connected to the sniffing port (Eltherm GmbH, Burbach, Germany), dividing the effluent into two equal parts. The same chromatographic conditions of the GC-MS analyses were used for the GC-O analyses, followed by an olfactory record of the odor-active volatile compounds.

#### 2.3.3. Aroma extract dilution analysis (AEDA)

A series of successive dilutions ( $2^n$ ) of the SAFE extract was prepared, and then, each solution was analyzed by GC-O on a DB-Wax column (Grosch, 1994; Schieberle, 1995). Three trained panelists from the Flavour Research and Education Center (The Ohio State University (Columbus, OH, USA) identified the odour-active zones. Each compound flavour dilution factor (FD) was established as the maximum dilution at which the panelists perceived an olfactory note in the sniffing port.

#### 2.3.4. Identification and quantitation of odour-active volatile compounds

The mass spectra of the compounds found to be significant contributors to *M. dubia* aroma were preliminarily compared with the NIST 2014 spectral library (Gaithersburg, MD). Then, the odour-active compounds were identified by comparison of their retention indexes (RI), mass spectra, and odour notes with those exhibited by standard solutions of the volatile compounds in DCM (30  $\mu\text{g}/\text{mL}$ ). A mixture of alkanes ( $\text{C}_7$ - $\text{C}_{30}$ ) was used to calculate the retention indexes.

The internal standard (IS) method was used to perform the quantitative analyses of odour-active volatiles IOFI, (2011). Lin-

**Table 1.** GC-MS data acquisition parameters for quantitation (MRM technique) of odour-active volatiles in camu-camu (*Myrciaria dubia*) fruit

No <sup>a</sup>	Compound	Transition (m/z) (Collision energy (eV))
1	(Z)-3-Hexen-1-ol	82→67(5)
2	Isoamyl acetate	70→55(5)
3	$\alpha$ -Pinene	93→77(10)
4	Limonene	107→79(2)
5	$\beta$ -Caryophyllene	133→105(5)

<sup>a</sup>Compounds were numbered according to Figure 1.

alool at a concentration of 250  $\mu\text{g/mL}$  was used as the internal standard (IS). The analyses were done in triplicate by constructing calibration curves varying the nominal concentrations of each analyte (IS:analyte from 1:5 to 5:1), assuming the slope as the response factor.

These analyses were performed using an Agilent 7890B GC (Agilent Technologies, Santa Clara, CA) equipped with a DB-5 column coupled to an Agilent 7010B Triple Quadrupole Mass Spectrometer (Agilent Technologies, Santa Clara, CA). The same chromatographic conditions described above were used. The MSD conditions were as follows: capillary transfer line and source temperature were set at 250 °C, mass range 30–350 u, and it was operated in electron ionization (EI) mode at 70 eV. Data were collected using multiple reaction monitoring mode (MRM). The MRM methods were optimized by injection of pure standards. Previously, each standard was analyzed in full scan mode (30–350 m/z) to identify precursor ions. Then, selected precursor ions were fragmented with different collision energies (2, 5, 10, 15, 20, 30, and 40 eV). Product ions were selected based on the abundance and selectivity of the transition from precursor to product ion for each standard (Table 1). For linalool, the transition parameters were 71.0→43.1 u at a collision energy of 5 eV. Finally, the concentrations for each odour-active compound were calculated, and the odor activity values (OAV) were determined as the relationship between the concentration and the threshold value in water taken from the literature. The quantitative data were employed to prepare the recombined solution of the fruit aroma profile.

### 2.3.5. Sensory analysis

The sensory evaluation was performed by an 11-judge trained panel (six women and five men, ranging in age from 21 to 50 years old) recruited from Disaromas S.A. (Bogotá, Colombia). During several sessions, sensory experiments were performed at 20  $\pm$  1 °C in a sensory room with single cabins. The judges were trained in orthonasal odor perception of descriptors, as well as managing intensity scales through reference solutions with the following aroma attributes: fruity (isoamyl acetate), green ((Z)-3-hexen-1-ol), herbal pine-tree-like ( $\alpha$ -pinene), citrus (limonene), and woody tree-bark-like ( $\beta$ -caryophyllene). For that purpose, a stock solution of each odorant in propylene glycol (10 mg/mL) was employed for preparing aqueous reference solutions, each at different concentrations between 10 and 90 times above the odor threshold of the respective odorant.

To prepare the fruit aroma recombined, appropriate amounts (10–200  $\mu\text{L}$ ) of the stock solutions of the odorants were mixed and made up to 1 L with water to yield the same concentrations as determined in *M. dubia* fruits. Fresh *M. dubia* pures (7 g) was

prepared under the same conditions as the isolated volatile extract. The fruit and the recombined mixture were placed in different amber glass bottles (20 mL each). Thus, assessors were asked to ortho-nasally evaluate the intensity of the five reference solutions in both samples using each linear 3-point attribute scale (0 = not perceivable; 1 = weakly perceivable; 2 = moderately perceivable; and 3 = strongly perceivable, Lawless and Heymann, 2010). Also, they compared the overall similarity of the aroma profile between the two samples. Reference solutions were presented at concentrations corresponding to the final point of scale (3). All samples were evaluated in three replicate sessions, and results were plotted in a spider web diagram.

### 2.4. Antihypertensive activity

Whole camu-camu fruits (100 g) were frozen at  $-4$  °C and lyophilized in a Beta 1-8 LDplus lyophilizer (CHRIST, Germany) for 48 h, with a main drying phase of 40 h at 1.0 mbar and  $-20$  °C. The final drying phase lasted eight h, at 0.001 mbar and  $-76$  °C. After freeze-drying, the seeds were manually extracted from the fruits; and the pulp and peel were homogenized using a grinder (A11 basic, IKA, Staufen, Germany), obtaining a fine powder. Camu-camu sample (peel and pulp) was prepared at 50  $\mu\text{g/mL}$ , where 500  $\mu\text{g}$  of fine powder was dissolved in 10 mL of deionized water, sonicated, and filtered using a PTFE syringe filter of 0.45  $\mu\text{m}$  (Agilent technologies, USA).

The *in vitro* antihypertensive activity of camu-camu, was determined by the ACE-I enzyme inhibition assay following the protocol provided by the manufacturer (Dojindo Molecular Technologies Inc, 2023). Commercial lisinopril was used as a positive control prepared at 50  $\mu\text{g/mL}$  in deionized water (ACE-I inhibition: 95.31  $\pm$  5.88 %). The absorbance of all samples was measured in a 96-well plate multireader using a BertholdTech TriStar2S spectrophotometer (Berthold Technologies GmbH & Co, KG, Germany), and the data was processed using ICE software, Version 1.0.9.0. The inhibition percentage of the samples was calculated considering the following equation

$$\text{ACE-I Inhibition (\%)} = \frac{A_{\text{Blank 1}} - A_{\text{Sample}}}{A_{\text{Blank 1}} - A_{\text{Blank 2}}} \times 100 \quad (1)$$

where, *A* is the absorbance value measured at  $\lambda$  450 nm, *Blank 1* is the positive control (no ACE inhibition), and *Blank 2* is the reagent blank.

### 2.5. Statistical analysis

Statistical analyses were performed using Statistica v12 (StatSoft) and InfoStat (Version 2020) software. All experiments were performed in triplicate by analysis of variance (ANOVA) and regression analysis, and average values were compared using Tukey's test with a probability  $p \leq 0.05$ . Data are presented as the mean and standard deviation of the three independent experiments.

## 3. Results and discussion

### 3.1. Odor-active compounds of camu-camu fruit

The volatile profile of *Myrciaria dubia* fruit was characterized by the presence of monoterpenes ( $\alpha/\beta$ -pinene, limonene,  $\beta$ -myrcene,  $\alpha$ -cymene, 3-carene,  $\alpha/\gamma$ -terpinene, and  $\alpha$ -phellandrene), sesquiter-

**Table 2. Odour-active volatiles identified in camu-camu (*Myrciaria dubia*) SAFE extract**

No. <sup>a</sup>	Odorant <sup>b</sup>	Odour description <sup>c</sup>	RI <sup>d</sup>		Concentration $\pm$ SD (%CV) <sup>e</sup>	Odour threshold ( $\mu\text{g}/\text{kg}$ water) <sup>f</sup>	OAV <sup>g</sup>
			DB-Wax	DB-5			
1	(Z)-3-Hexen-1-ol	Green	1384	849	1.0 $\pm$ 0.9 (8.8%)	70	<1
2	Isoamyl acetate	Fruity, sweet	1131	873	22.1 $\pm$ 7.6 (9.1%)	2	11.1
3	$\alpha$ -Pinene	Pine, herbal	1035	942	3038.3 $\pm$ 13.4 (1.7%)	6	506.4
4	Limonene	Citrus, fruity	1216	1042	7814.9 $\pm$ 16.9 (2.1%)	10	781.4
5	$\beta$ -Caryophyllene	Woody, tree bark, Terpeny, earthy	1630	1449	4271.1 $\pm$ 14.7 (0.3%)	64	66.7

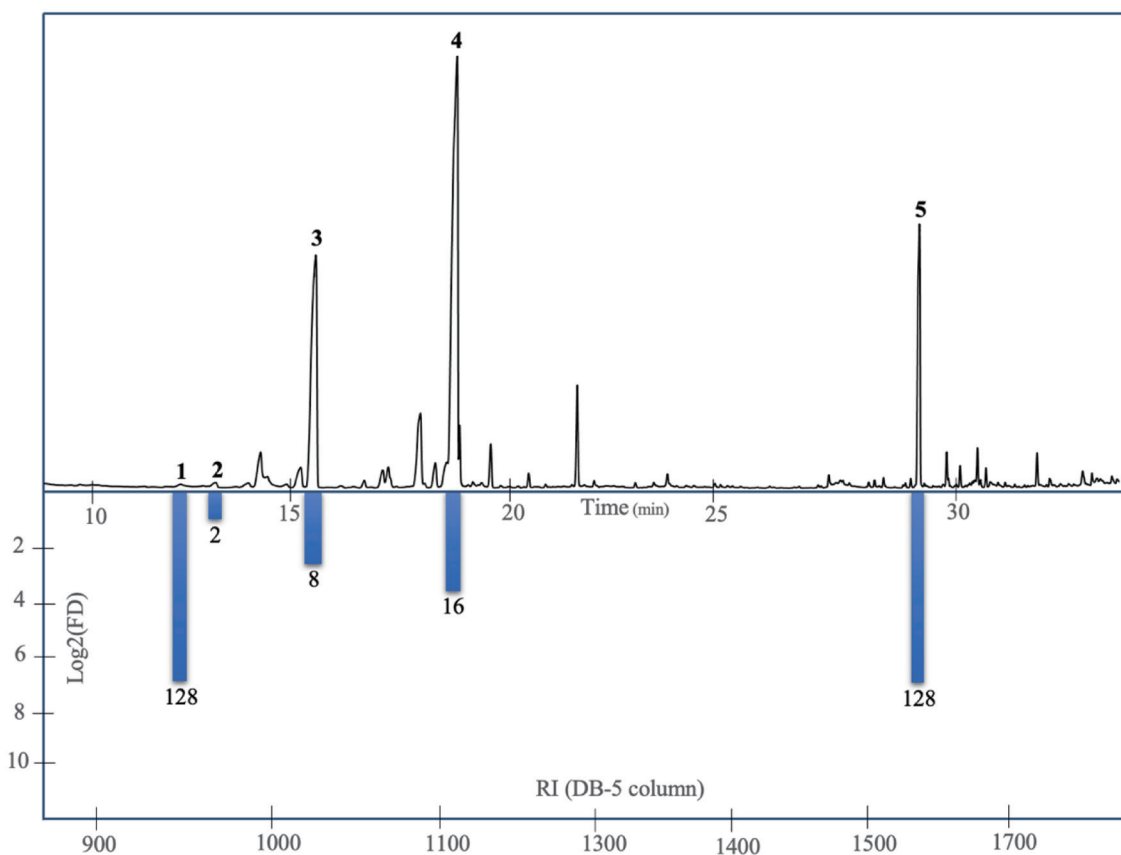
<sup>a</sup>Odorants were numbered according to their retention time on DB-5 column (Figure 1). <sup>b</sup>Odorants were identified by comparing their retention indices on the DB-Wax and DB-5 column, their mass spectra, and their odor notes (detected by GC-O) with respective data of reference compounds. <sup>c</sup>Odour note as perceived at the sniffing port during GC-O analysis. <sup>d</sup>Retention index. <sup>e</sup>All data are the mean of three measurements  $\pm$  standard deviation and coefficient of variation (CV). In all the cases, the correlation coefficient was higher than 0.995. <sup>f</sup>Leffingwell (2008). <sup>g</sup>OAV = Odour Activity Value, concentration divided by their odor threshold.

penes ( $\beta$ -caryophyllene,  $\alpha$ -humulene,  $\gamma$ -elemene, and  $\alpha$ -calacorene), monoterpenols (terpinen-4-ol and  $\alpha$ -terpineol), aliphatic alcohols (isoamyl alcohol, butanol, and (Z)-3-hexen-1-ol), esters (isoamyl acetate, 2-ethyl furoate, ethyl benzoate, and ethyl butyrate), and the aldehyde nonanal. Most of these compounds were also reported by Franco and Shibamoto (2000); however, *o*-cymene, isoamyl acetate, and isoamyl alcohol were here identified as volatile constituents of *M. dubia* fruit. The use of solvent extraction with a more polar solvent (dichloromethane) rather than the retention in Porapak Q trap and hexane elution used by Franco and Shibamoto (2000), allowed here to isolate the isoamyl acetate that is one of the odour-active

volatile compounds in *M. dubia* fruit.

By using the molecular sensory approach, this study successfully identified the odour-active volatiles in the aroma of *M. dubia* fruit. After analyzing the SAFE volatile extract using GC-MS/GC-O and AEDA analysis, only (Z)-3-hexen-1-ol, isoamyl acetate,  $\alpha$ -pinene, limonene, and  $\beta$ -caryophyllene were identified as odour-active volatile compounds in this fruit (Table 2). The AEDA analysis (Figure 1) showed that (Z)-3-hexen-1-ol and  $\beta$ -caryophyllene were the volatile compounds with the highest FD compared to the other compounds in the extract.

The quantitation experiments were done by using the MRM



**Figure 1. GC and AEDA analyses on DB-5 column of odour-active compounds from *Myrciaria dubia* fruit. Peak numbers correspond to the compound numbers in Table 2.**

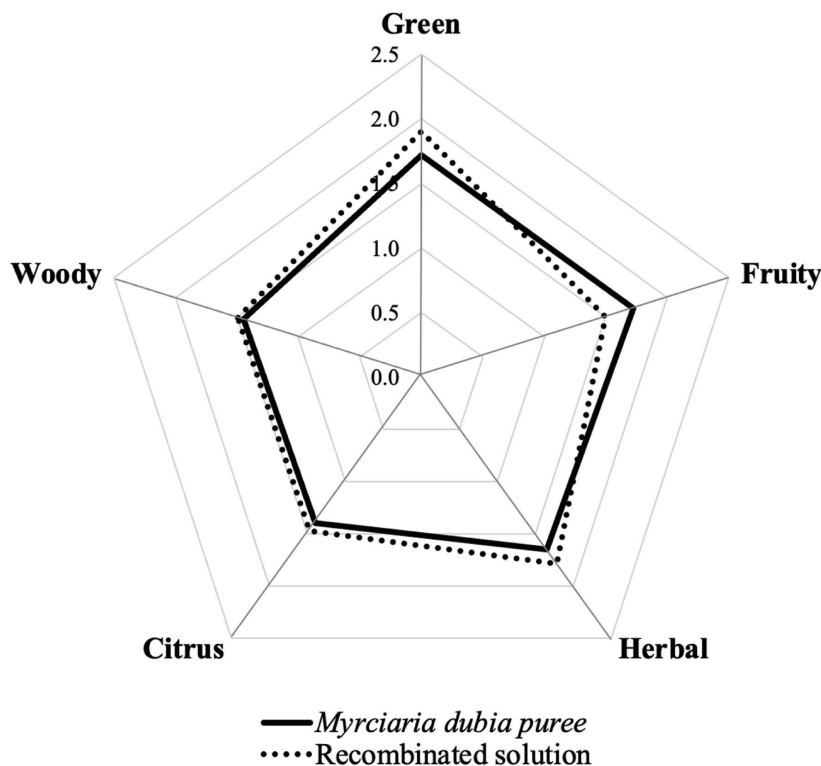


Figure 2. Comparative aroma sensory profiles of *M. dubia* fruit and aroma recombined solution.

technique with linalool as the internal standard. The MS parameters such as precursor and product ions are presented in Table 1. The OAVs were calculated for each compound, finding that *a*-pinene and limonene were the odour-active volatiles with the highest contribution to the aroma of this fruit. This finding agrees with the fact that terpenes are the main class of volatile compounds in different maturation stages of this fruit (Souza et al., 2021). It is noteworthy that *a*-pinene and limonene have also been reported as odour-active compounds in citrus fruit, such as clementine (Buettner et al. 2003) and orange (Hinterholzer and Schieberle, 1998).

An aroma recombination sample was prepared using the natural quantities found for each of the odorants listed in Table 2 and evaluated in comparison with the fruit puree (pulp and peel) by the sensory panel (Figure 2). Taking into account that the moisture content of fresh *M. dubia* fruit is above 90%, water was chosen as an appropriate matrix for the recombination experiments, and also was considered an odorless material. In general, trained judges agreed with the odor similarity between the reconstituted mixture and fresh fruit, determining more juiciness in the fruit sample. As (*Z*)-3-hexen-1-ol presented OAV < 1, it was not included in the preparation of the reconstituted mixture. However, the green note was evaluated in the recombinant experiments because it was perceived by the judges in both, the mixture and the fruit. Previous sensory evaluations of camu-camu juice from pulp reported woody, citrus, herbal, spicy, and camphoraceous notes in the aroma description (Campelo et al., 2020). Nonetheless, the presence of the peel in all of the experiments here performed likely contributed to reported different odour descriptors. The peel was included for better use of the fruit and reduction of residues after processing. In general, the intensities of the citrus, herbal, and woody (attributed to  $\beta$ -caryophyllene, *a*-pinene, and limonene, respectively) odor attributes in the aroma recombination sample were similar to those of the fruit puree. In contrast,

the fruity note was rated lower than the original sample. Actually, judges associated the fruity-citrus notes with lemon peel. Because the aroma recombination sample closely matched the fruit aroma profile, it can be assumed that the key aroma compounds in camu-camu were satisfactorily characterized.

The volatile profile of *M. dubia* was similar to that of other Myrtaceae fruits from the Amazon region, such as jaboticaba (*Myrciaria jaboticaba*), where terpenes ( $\beta$ -pinene and  $\delta$ -cadinene) and alcohols (2-phenylethanol and linalool) were identified as the major volatile constituents (Plagemann et al., 2012). Guavaberry (*Myrciaria floribunda*) reported numerous sesquiterpenes with caryophyllene and  $\gamma$ -selinene being the most predominant volatile constituents (Mendoza García et al., 2021). In cagaita pulp (*Eugenia dysenterica*) *a*-pinene, (*Z*)- $\beta$ -ocimene, and *a*-terpineol were the major constituents (Dos Santos Dias et al., 2021), whereas uvaiva pulp (*Eugenia pyriformis*) exhibited different terpenes in the volatile profile (Fernandes de Araújo et al., 2019). Likewise, the volatile profile of araza (*Eugenia stipitata*) consisted of  $\beta$ -ocimene as the major volatile compound (Fernandes de Araújo et al., 2021); and in pitanga fruit (*Eugenia uniflora* L.) the volatile composition in four ripening stages was predominantly composed by terpenoid compounds (Santos Silva et al., 2019). These findings suggest that the 1-deoxy-D-xylulose 5-phosphate (DXP) biogenetic pathway is highly activated from the production of terpenes (Souza et al., 2021). Similarly, various reports suggested that the formation of terpenes in Myrtaceae species is primarily attributed to metabolic pathways that begin with the geranyl and farnesyl cations, leading to monoterpenes and sesquiterpenes transformations (Silva da Costa et al., 2022). Terpenes are some of the most prevalent and structurally varied secondary metabolites in plants that enable interactions between plants and their surroundings, which are essential for all stages of their life cycle. Moreover, terpenes are also

recognized for having biological and functional properties in food, pharmaceutical, cosmetic, and fragrance applications. In the food industry, terpenes have been used to add flavour and aroma to different food products, allowing the development of emerging food designs and trends. Additionally, terpenes are distinguished for exhibiting therapeutic and bioactive applications since they present anticancer, antibacterial, antihyperglycemic, anti-inflammatory, antioxidant, immunomodulatory, and antiviral activity, providing health benefits for the prevention and treatment of some diseases (Brahmkshatriya and Brahmkshatriya, 2013).

### 3.2. Antihypertensive activity

The freeze-dried Amazonian camu-camu fruit was assessed under ACE-I enzyme activity inhibition assay, which is used to determine the *in vitro* antihypertensive activity. The ACE-I inhibition is one of the most employed strategies to prevent and manage hypertension, since it could prevent or delay the onset of high blood pressure and reduce side effects even from non-communicable diseases (Felkle et al., 2022). In plant materials, the capacity to inhibit ACE-I enzyme is usually given by its chemical composition, mainly constituted by vitamins, minerals, and dietary polyphenols. In fact, phenolic compounds such as flavonoids, and ellagitannins in berries like strawberry (*Fragaria × ananassa*), blueberry (*Vaccinium corymbosum*), or raspberry (*Rubus idaeus*), and seems to have played an important role in the modulation of chronic hypertension (Yousefi et al., 2020).

The ACE-I inhibition activity exhibited by the *M. dubia* fruit was  $39.48 \pm 12.09\%$  at the endogenous fruit concentration (50 µg/mL), with lisinopril used as the positive control. This is a promising result, considering that Fujita et al. (2015) did not report any enzymatic ACE-I inhibition for *M. dubia* pulp, even at a sample concentration 200 times higher (10 mg/mL) than the one employed in this assay. Furthermore, the jaboticaba (*Myrciaria jaboticaba*) seed extract inhibited the ACE-I enzyme ( $22.4 \pm 0.2\%$ ), which was attributed mainly to the hydroxyl groups in the benzene ring of the phenolic compounds such as castalagin, procyanidin A2, and ellagic acid (Fidelis et al., 2021).

The polyphenols previously detected in *M. dubia* pulp powder obtained by spray-drying were the flavonol myricetin and its conjugates ( $3.05 \pm 0.07$  mg/100 g powder), ellagic acid derivatives ( $9.75 \pm 0.10$  mg/100 g powder), ellagitannins ( $16.10 \pm 0.33$  mg/100 g powder), and anthocyanins ( $19.63 \pm 0.60$  mg/100 g powder), being 2-fold concentration higher in the fresh peel than in the fresh pulp (Fracasetti et al., 2013). Moreover, the main phenolic compounds identified in *M. dubia* peels were myricetin-*O*-pentoside ( $4.91 \pm 0.08$  mg/g extract) and cyanidin-3-*O*-glucoside ( $4.68 \pm 0.07$  mg/g extract). Taking into account that *M. dubia* peel ( $33.4 \pm 0.5$  mg/g extract) exhibited a higher polyphenolic content than the pulp ( $4.32 \pm 0.03$  mg/g extract) (Conceição et al., 2020), the results here obtained potentially can be attributable to the polyphenols present in the *M. dubia* peel.

It has been reported that polyphenols block ACE-I enzyme by filling the bioactive sites. Specifically, flavan-3-ols and procyanidins have an inhibitory effect on the ACE-I activity, which depends on the number of epicatechin units of procyanidin (Actis-Goretta et al., 2003). Phenolic compounds inhibit ACE-I enzyme by generating chelates with the zinc atom within the active center, among other proposed mechanisms (Chen et al., 2021).

## 4. Conclusion

The sensomics approach allowed the identification of the four vol-

atile compounds that contributed to the aroma of the *M. dubia* fruit. Likewise, the sensory evaluation showed the relevance of terpenes ( $\alpha$ -pinene, limonene, and  $\beta$ -caryophyllene) in the aroma of this fruit, exhibiting herbal, woody, and citrus odour notes. Moreover, the camu-camu fruit (pulp and peels) showed an *in vitro* antihypertensive activity by inhibiting the ACE-I enzyme activity. These results show that camu-camu fruit is a promising raw material for developing added-value products with bioactive properties based not only on vitamin C but also on the polyphenol content, allowing sustainable exploitation of this Amazonian fruit. With these results, the medium-term objective is to increase consumer awareness of this native and tropical Amazonian fruit and evaluate its potential role as raw material for functional foods.

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## Author note

This work is dedicated to the memory of Prof. Dr. Michael Granvogl. He was a scientist full of life, with excellent contributions to the flavour chemistry field, and an FC Bayern München fan with good vibes.

## Conflict of interest

The authors declare no conflict of interest. The founding sponsors were not involved in planning the study, data collecting, analysis, interpretation, manuscript writing, or the choice to publish the findings.

## References

- Actis-Goretta, L., Ottaviani, J.I., Keen, C.L., and Fraga, C.G. (2003). Inhibition of angiotensin converting enzyme (ACE) activity by flavan-3-ols and procyanidins. *FEBS Lett.* 555: 597–600.
- Brahmkshatriya, P.P., and Brahmkshatriya, P.S. (2013). Terpenes: Chemistry, biological role, and therapeutic applications. In: Ramawat, K., and Mérillon, J.M. (Ed.). *Natural Products*. Springer, Berlin, Heidelberg, Germany, pp. 2665–2691.
- Buettner, A., Mestres, M., Fischer, A., Guasch, J., and Schieberle, P. (2003). Evaluation of the most odour-active compounds in the peel oil of clementines (*Citrus reticulata* blanco cv. clementine). *Eur. Food Res. Technol.* 216: 11–14.
- Campelo, P.H., Alves Filho, E.G., Silva, L.M.A., de Brito, E.S., Rodrigues, S., and Fernandes, F.A.N. (2020). Modulation of aroma and flavor us-

- ing dielectric barrier discharge plasma technology in a juice rich in terpenes and sesquiterpenes. *LWT-Food Sci. Technol.* 130: 109644.
- Castro, J.C., Maddox, J.D., Cobos, M., Paredes, J.D., Marapara, J.L., Braga, J., Imán, S.A., Rodríguez, H.N., and Castro, C.G. (2020). Bioactive compounds of Camu-Camu (*Myrciaria dubia* (Kunth) McVaugh). Bioactive Compounds in Underutilized Fruits and Nuts. Reference Series in Phytochemistry. Springer, Cham, Switzerland, pp. 329–352.
- Chen, L., Wang, L., Shu, G., and Li, J. (2021). Antihypertensive potential of plant foods: Research progress and prospect of plant-derived angiotensin-converting enzyme inhibition compounds. *J. Agric. Food Chem.* 69: 5297–5305.
- Conceição, N., Albuquerque, B.R., Pereira, C., Corrêa, R.C.G., Lopes, C.B., Calheta, R.C., Alves, M.J., Barros, L., and Ferreira, I.C.F.R. (2020). By-products of camu-camu [*Myrciaria dubia* (Kunth) McVaugh] as promising sources of bioactive high added-value food ingredients: Functionalization of yogurts. *Molecules* 25(1): 70.
- Cunha-Santos, E.C.E., Viganó, J., Neves, D.A., Martínez, J., and Teixeira Godoy, H. (2019). Vitamin C in camu-camu [*Myrciaria dubia* (H.B.K.) McVaugh]: evaluation of extraction and analytical methods. *Food Res. Int.* 115: 160–166.
- Dojindo. ACE Kit – WST Technical Manual. [https://www.dojindo.eu.com/TechnicalManual/Manual\\_A502.pdf](https://www.dojindo.eu.com/TechnicalManual/Manual_A502.pdf). Accessed March.
- Dos Santos Dias, E.B., Vargas Camilo, Y.M., Barboza de Souza, E.R., and Ferri, P.H. (2021). Essential oil variability in *Eugenia dysenterica* fruits. *Nat. Prod. Res.* 6: 1–4.
- Engel, W., Bahr, W., and Schieberle, P. (1999). Solvent assisted flavour evaporation - a new and versatile technique for the careful and direct isolation of aroma compounds from complex food matrices. *Eur. Food Res. Technol.* 209: 237–241.
- Felkle, D., Jarczyński, M., Kaleta, K., Zięba, K., and Nazimek, K. (2022). The immunomodulatory effects of antihypertensive therapy: A review. *Biomed. Pharmacother* 153: 113287.
- Fernandes de Araújo, F., Neri-Numa, I.A., de Paulo Farias, D., Castro da Cunha, G.R.M., and Pastore, G.M. (2019). Wild Brazilian species of *Eugenia* genera (Myrtaceae) as an innovation hotspot for food and pharmacological purposes. *Food Res. Int.* 121: 57–72.
- Fernandes de Araújo, F., de Paulo Farias, D., Neri-Numa, I.A., Dias-Audibert, F.L., Delafiori, J., Gama de Souza, F., Ramos Catharino, R., Kersul do Sacramento, C., and Pastore, G.M. (2021). Chemical characterization of *Eugenia stipitata*: A native fruit from the Amazon rich in nutrients and source of bioactive compounds. *Food Res. Int.* 139: 109904.
- Fidelis, M., Sousa Santos, J., Bragueto Escher, G., Vieira do Carmo, M., Azevedo, L., da Silva, M.C., Putnik, P., and Granato, D. (2018). *In vitro* antioxidant and antihypertensive compounds from camu-camu (*Myrciaria dubia* McVaugh, Myrtaceae) seed coat: A multivariate structure-activity study. *Food Chem Toxicol* 120: 479–490.
- Fidelis, M., Santos, J.S., Escher, G.B., Rocha, R.S., Cruz, A.G., Cruz, T.M., Marques, M.B., Nunes, J.B., do Carmo, M.A.V., de Almeida, L.A., Kaneshima, T., Azevedo, L., and Granato, D. (2021). Polyphenols of jaboticaba [*Myrciaria jaboticaba* (Vell.) O.Berg] seeds incorporated in a yogurt model exert antioxidant activity and modulate gut microbiota of 1,2-dimethylhydrazine-induced colon cancer in rats. *Food Chem.* 334: 127565.
- Fracassetti, D., Costa, C., Moulay, L., and Tomás-Barberán, F.A. (2013). Ellagic acid derivatives, ellagitannins, proanthocyanidins and other phenolics, vitamin C and antioxidant capacity of two powder products from camu-camu fruit (*Myrciaria dubia*). *Food Chem.* 139(1-4): 578–588.
- Franco, M.R.B., and Shibamoto, T. (2000). Volatile composition of some Brazilian fruits: Umbu-caja (*Spondias citifera*), camu-camu (*Myrciaria dubia*), araçá-boi (*Eugenia stipitata*), and cupuaçu (*Theobroma grandiflorum*). *J. Agric. Food Chem.* 48: 1263–1265.
- Fujita, A., Sarkar, D., Wu, S., Kennelly, E., Shetty, K., and Genovese, M.I. (2015). Evaluation of phenolic-linked bioactives of camu-camu (*Myrciaria dubia* McVaugh) for antihyperglycemia, antihypertension, antimicrobial properties and cellular rejuvenation. *Food Res. Int.* 77(Part 2): 194–203.
- García-Chacón, J.M., Tello, E., Coy-Barrera, E., Peterson, D.G., and Osorio, C. (2022). Mono-*n*-butyl malate-derived compounds from Camu-camu (*Myrciaria dubia*) Malic Acid: The Alkyl-dependent antihyperglycemic-related activity. *ACS Omega.* 7(43): 39335–39346.
- Granvogl, M., and Schieberle, P. The sensomics approach: a useful tool to unravel the genuine aroma blueprint of foods and aroma changes during food processing. In: Barceló, D. (Ed.). *Comprehensive Analytical Chemistry*. Wilson & Wilson, Amsterdam, Netherlands, pp. 41–68.
- Grosch, W. (1994). Determination of potent odorants in foods by Aroma Extract Dilution Analysis (AEDA) and calculation of odour activity values (OAVs). *Flavour Fragr. J.* 9: 147–158.
- Hinterholzer, A., and Schieberle, P. (1998). Identification of the most odour-active volatiles in fresh, hand-extracted juice of Valencia late oranges by odour dilution techniques. *Flavour Fragr. J.* 13: 49–55.
- IOFI Working Group on Methods of Analysis. (2011). Guidelines for the quantitative gas chromatography of volatile flavouring substances, from the Working Group on Methods of Analysis of the International Organization of the Flavor Industry (IOFI). *Flavour Fragr. J.* 26: 297–299.
- Leffingwell & Associates. (2008). Odor detection thresholds and references. <http://www.leffingwell.com/odorthre.htm>. Accessed March 2023.
- Lawless, H.T., and Heymann, H. *Descriptive Analysis. Sensory Evaluation of Food.* Food Science Text Series. Springer, New York, NY, pp. 227–257.
- Mendoza García, Y., Cruz Ramos, A.L.C., Cardoso Clemente Filha Ferreira de Paula, A., do Nascimento, M.H., Augusti, R., Linhares Bello de Araújo, R., Pinto de Lemos, E.E., and Ferreira Melo, J.O. (2021). Chemical physical characterization and profile of fruit volatile compounds from different accesses of *Myrciaria floribunda* (H. West Ex Wild.) O. Berg through polyacrylate fiber. *Molecules* 26(17): 5281.
- Neves, L.C., da Silva, V.X., Alves Pontis, J., Flach, A., and Roberto, S.R. (2015). Bioactive compounds and antioxidant activity in pre-harvest camu-camu [*Myrciaria dubia* (H.B.K.) Mc Vaugh] fruits. *Sci. Hortic.* 186: 223–229.
- Plagemann, I., Krings, U., Berger, R.G., and Marostica, M.R. Jr (2012). Volatile constituents of jaboticaba (*Myrciaria jaboticaba* (Vell.) O. Berg) fruits. *J. Essent. Oil Res.* 24(1): 45–51.
- Santos, I.L., Miranda, L.C.F., da Cruz Rodrigues, A.M., da Silva, L.H.M., and Amante, E.R. (2022). Camu-camu [*Myrciaria dubia* (HBK) McVaugh]: A review of properties and proposals of products for integral valorization of raw material. *Food Chem.* 372: 131290.
- Santos Silva, J., Damiani, C., da Cunha, M.C., Nunes Carvalho, E.E., and de Barros Vilas Boas, E.V. (2019). Volatile profiling of pitanga fruit (*Eugenia uniflora* L.) at different ripening stages using solid-phase microextraction and mass spectrometry coupled with gas chromatography. *Sci. Hortic.* 250: 366–370.
- Silva da Costa, J., Andrade, W.M.S., de Figueiredo, R.O., Santos, P.V.L., da Silva Freitas, J.J., Setzer, W.N., da Silva, J.K.R., Maia, J.G.S., and Figueiredo, P.L.B. (2022). Chemical composition and variability of the volatile components of *Myrciaria* species growing in the Amazon region. *Molecules* 27: 2234.
- Schieberle, P. In: Gaonkar, A.G. (Ed.). *Recent developments in methods for analysis of flavor compounds and their precursors.* Elsevier, Amsterdam, The Netherlands, pp. 403–431.
- Souza, F. das C. do A., Silva, E.P., and Aguiar, J.P.L. (2021). Vitamin characterization and volatile composition of Camu-Camu (*Myrciaria Dubia* (HBK) McVaugh, Myrtaceae) at different maturation stages. *Food Sci. Technol.* 41(4): 961–966.
- WHO – World Health Organization. Hypertension. <https://www.who.int/news-room/fact-sheets/detail/hypertension>. Accessed February 2023.
- Yousefi, M., Shadnoush, M., Khorshidian, N., and Mortazavian, A.M. (2020). Insights to potential antihypertensive activity of berry fruits. *Phytother. Res.* 35(2): 846–863.